Acanthobrama terraesanctae (YARON[8]) and not in the thecal cells, as reported by BARA[9] in the ovary of *Scomber scomber*. In *Monopterus albus*, the presence of *3β*-HSD activities in the granulosa cells of the ovarian follicles albeit only in the active period of the sexual cycle provides strong evidence that these cells are capable of producing sex steroids. This, together with the previous findings that oestrogens were produced by the *Monopterus* ovary in vitro (CHAN and PHILLIPS[2]), indicates that the granulosa cells are the sites of oestrogen production. Negative reactions for 3β-HSD in ovaries during other parts of the sexual cycle and for 17β-HSD throughout the whole year may possibly be due to the limitation in the sensitivity of the histochemical technique employed. The demonstration of 3β-HSD in the breeding season is perhaps associated with the increase in the production of oestrogen, required for vitellogenesis.

3β-HSD activities were also found in the interstitial cells of some *Monopterus* ovaries where these cells were present. Interstitial cell activities for 3β-HSD in other teleostean ovaries seem to have only been reported in *Cymatogaster aggregata* (WEIBE[10]). Whether these cells secrete oestrogens or androgens remains unknown. So far no work has been done on the steroid enzyme histochemistry in any fish that undergoes natural sex reversal. The present study indicated that the *Monopterus* ovary possesses the enzymes essential for steroidogenesis. The significance of this is at present under investigation.

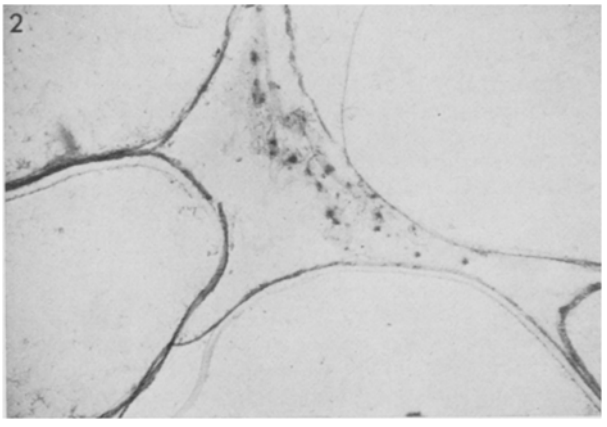

Fig. 2. Part of the ovary showing 3β-hydroxysteroid dehydrogenase activities in the granulosa cells and in the interstitial cells. May sample. 20 μm. ×28.

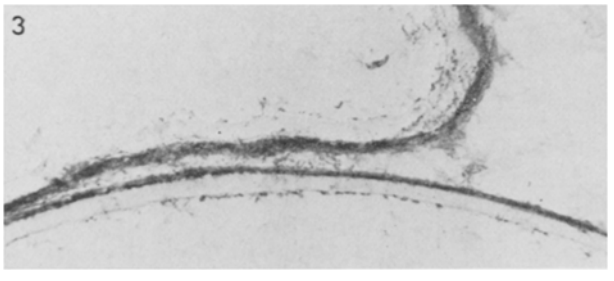

Fig. 3. Detail of the follicular epithelium of a mature follicle showing 3β-hydroxysteroid dehydrogenase activities in the granulosa cells. 20 μm. ×80.

Résumé. L'histochimie des enzymes de l'ovaire de *Monopterus* révèle la présence d'activités deshydrogénase hydroxystéroïde-3β dans les cellules granuleuses des grands follicules pendant la saison de la reproduction. Cela indique que les cellules granuleuses de ces follicules peuvent produire des steroïdes, peut-être des oestrogènes. Des cellules interstitielles de l'ovaire de *Monopterus* présentent aussi des réactions de deshydrogénase hydroxysteroïde-3β positives, mais il est impossible de décider si ces cellules produisent des androgènes ou des oestrogènes.

F. TANG[11], B. LOFTS and S.T.H. CHAN

Department of Zoology,
University of Hong Kong,
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[11] Present address: Department of Zoology, University of Hull, Hull HU6 7RX (England).

PRO EXPERIMENTIS

A New Colorimetric Method for the Assay of the Serum Glyoxalase System

The glyoxalase system consists of 2 enzymes: glyoxalase I (*S*-lactoylglutathione methylglyoxal-lyase E.C. 4.4.1.5) which produces lactoylglutathione, and glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase E.C. 3.1.2.6) which splits off free glutathione. The reactions catalyzed by the 2 enzymes can be written:

$$CH_3COCHOHSG \underset{\text{(I)}}{\rightleftharpoons} CH_3CHOHCOSG$$

$$CH_3CHOHCOSG + H_2O \underset{\text{(II)}}{\rightleftharpoons} CH_3CHOHCOOH + GSH$$

Sum:

$$CH_3COCHOHSG + H_2O \rightleftharpoons CH_3CHOHCOOH + GSH$$

The first substrate is formed spontaneously from methylglyoxal and glutathione (GSH) in a reaction with a K_s value of 2×10^{-3} *M*. The reaction (I), catalyzed by glyoxalase I, has a K_m value of 0.19×10^{-3} *M* for the hemimercaptal, as shown with porcine erythrocytes enzyme by MANNERVIK et al.[1]. The importance of the glyoxalate system in cell growth regulation has been discussed extensively by FRENCH and FREEDLANDER[2] and by EYGUD and SZENT-GYORGYI[3]. The assays, used and described by RACKER[4], include a spectrophotometric technique at 240 nm, a manometric method and a colorimetric method based on hydroxamic acid formation.

Using the –SH group reagent of ELLMAN[5] we have developed a continuous spectrophotometric technique the rate of change of absorbance (Δ_2A/min) again recorded. The difference (Δ_2A/min − Δ_1A/min = ΔA/min) re-

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for studying the hemimercaptal splitting activity of human serum, in a reaction catalyzed by glyoxalase II and rate limited by the isomerizing enzyme glyoxalase 1. The system which proved to be most suitable contained 37.5 μmole of phosphate buffer pH 7.4, 20 μmole of $MgCl_2$, 58.2 μmole of methylglyoxal (Fluka, Buchs, Switzerland), 10 μmole of reduced glutathione (B.D.H., Poole, England) and 2 μmole of 2′, 2′ dithiobisnitrobenzoic acid (Fluka, Buchs, Switzerland) in a total volume of 2.4 ml. The system was incubated at 25 °C for 2 min and the rate of change of absorbance at 412 nm (Δ_1A/min) was recorded with an UNICAM SP 1800 recording spectrophotometer. Then 0.1 ml of serum was added, and presents the velocity of the enzymatic reaction. The order of addition of the reagents must be considered critical and the change of absorbance after the addition of serum was measured as a tangent of the initial part of the curve that gives the enzymatic reaction kinetic. The extinction coefficient of free GSH was measured with the complete system but without methylglyoxal, and a factor of conversion of 1400 (S.D. ± 120) was calculated. The factor is to transform the value of ΔA/min into International Units (I.U. = μmole of GSH liberated. $min^{-1} \times l^{-1}$ at 25 °C).

Table I. Velocity of SH group liberation with different concentrations of serum

Serum volume (ml)	ΔA/min (± S.D.)
0.012	0.005 ± 0.001
0.020	0.013 ± 0.002
0.040	0.017 ± 0.004
0.080	0.040 ± 0.010
0.100	0.050 ± 0.013
0.150	0.063 ± 0.015
0.200	0.072 ± 0.019

The medium was composed of 1.5 ml of 0.025 *M* phosphate buffer pH 7.4, 0.2 ml of 0.1 *M* $MgCl_2$, 0.4 ml of 0.01 *M* methylglyoxal, 0.1 ml of 0.1 *M* glutathione, 0.2 ml of 0.01 *M* ELLMAN's reagent[5] and different amounts of serum. The final volume was always 2.6 ml and the results are an average of 10 experiments carried out with 10 different normal sera.

Table II. Effect of different compounds on the percent activity of the glyoxalase system

Compound	Percent activity
None	100
− Mg	67
+ Colchicine	59
+ Eserine	100
+ EDTA	73
+ *o*-Phenantroline	36

The conditions are similar to those given in Table I. The compounds used are 1×10^{-3} *M* colchicine (Monks, Nairobi, Kenya), 0.7×10^{-3} *M* eserine (Fluka, Buchs, Switzerland), 0.75×10^{-3} *M* EDTA (B.D.H., Poole, England), 0.5×10^{-3} *M* *o*-phenantroline (B.D.H., Poole, England). All the concentrations given are final ones and the data are average values from experiments. An activity of 100 corresponds to a ΔA/min of 0.050.

Table III. SH group liberation due to the activity of glyoxalase system in serum

Subjects	Number	I.U.	Standard deviation	*t*	*p*
Normal	25	75.0	± 49.0	–	–
Cancer patients	25	120.0	± 70.0	2.66	0.01

The determinations were run in duplicate on fresh blood serum, and a correction for the absorbance change due to the titration of the free SH groups present in the serum and independent from the glyoxalase system was always applied.

The effect of different amounts of serum on the velocity of the reaction was checked, and the results are summarized in Table I. A linear relationship between volume of serum and change of absorbance/min at 412 nm holds up to 0.1 ml of serum. The enzymatic activity increases when the concentration of substance is increased, up to an optimal concentration of 2 to 5×10^{-3} *M* for GSH and 22 to 24×10^{-3} *M* for methylglyoxal. The apparent K_m value calculated for GSH with the double reciprocal method was 1.5×10^{-3} *M*, which, by assuming a value of 2×10^{-3} *M* as the dissociation constant for the hemimercaptal[1], indicates an apparent K_m value of 1.2×10^{-2} *M* for the enzymatic hydrolysis of the substrate hemimercaptal. Concentrations of methylglyoxal and GSH higher than that indicated as the optimal ones were inhibitory, as observed by other authors with different methods of assay[6].

The effect of Mg ions and some possible inhibitors was also checked and the results are summarized in Table II. The dependance of the present assay on the activity of the glyoxalase I is evident from the action of the inhibitors of this enzyme and from the need of Mg ions for full activity. The lack of inhibition by eserine suggests, on the other hand, that the hydrolytic activity is not due to aspectific esterases and therefore the assay can give a measure of the functioning of the whole glyoxalase system in the serum.

A preliminary analysis of the serum activity in a group of normal subjects of both sexes, aged between 21 and 46, was carried out and the results compared with those obtained from a group of patients suffering from cancer (mainly breast and prostate cancer). Table III summarizes the results.

The statistical significance of the difference in the activity of the glyoxalase system between normal individuals and cancer patients seems to provide some support for the hypothesis of SZENT-GYORGYI[7], and it is interesting to note that increase in glyoxalase activity has also been observed as a very early alteration in animals with actively regenerating tissues[8].

Zusammenfassung. Eine Methode für die Bestimmung der Serumglyoxalase wird beschrieben und die Kinetik der Hydrolyse von Lactoylglutathion mittels des Anwachsens freier SH-Gruppen gemessen. Eine Gruppe normaler Kontrollpersonen wurde mit einer Gruppe krebskranker Patienten verglichen. Im Serum der Krebspatienten wurde erhöhte Glyoxalaseaktivität gefunden.

L. GALZIGNA, H. S. NYANDIEKA and A. BURLINA

Department of Medical Biochemistry, University of Nairobi, P.O. Box 30197, Nairobi (Kenya), 20 July 1973.

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